Reference: *Biol. Bull.* **151**: 601–614. (December, 1976)

OSMOTIC ADJUSTMENT IN AN ESTUARINE POPULATION OF *UROSALPINX CINEREA* (SAY, 1822) (MURICIDAE, GASTROPODA)

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Marine and estuarine molluscs have long been considered to be strict osmotic conformers with a limited degree of ionic regulation (Prosser and Brown, 1961; Potts and Parry, 1964). However, studies by Todd (1964) and Peterson and Duerr (1969) suggest that this generalization may have to be modified in light of their experimental findings. Unfortunately, neither study yielded definitive results, and the question of possible osmotic regulation for some species of marine and estuarine molluscs is still unanswered.

Todd (1964) demonstrated that marsh periwinkles, *Littorina littorea*, held in sea water of 8.8‰ salinity had blood osmolalities as much as three times greater than that of the external medium. These results as indicators of osmotic regulation are debatable, since *L. littorea* is capable of completely sealing itself off from the environment by withdrawing into the shell and plugging the shell aperture with its operculum. Thus, it is conceivable that the test animals were not responding physiologically to the salinity but had simply "isolated" themselves from it through a morphological-behavioral adaptation. This shutting off from the environment is typical of many intertidal molluscs and serves to prevent desiccation when the animals are exposed to the atmosphere during periods of low tide.

Peterson and Duerr (1969) suggested osmotic regulation in *Tegula funebralis*, an intertidal limpet, on the basis of a decrease in free amino acid content of the tissue when the test animals were transferred from water having a salinity 120% that of normal sea water to 160% strength. Their contention of osmotic regulation was based on the known role of free amino acids and other small organic ions as osmotic effectors of intracellular fluids of marine and estuarine invertebrates (Duchâteau, Sarlet, Camien and Florkin, 1952; Lewis, 1952; Duchâteau and Florkin, 1955, 1956; Potts, 1958; Shaw, 1958; Simpson, Allen and Awapara, 1959; Allen, 1961; Awapara, 1962; Lange, 1963; Lynch and Wood, 1966; Schoffeniels, 1967). However, they did not investigate salinity-induced changes of those inorganic ions which are major osmotic effectors of both intracellular and extracellular body fluids (Potts and Parry, 1964; Robertson, 1964). Also, the decrease in free amino acid content was not accompanied by a decrease in total ninhydrin-positive substances indicating that other, unidentified organic ions varied directly with salinity. The observed inverse relationship between salinity and free amino acid content does not, by itself, lend much support to their contention of osmotic regulation in *T. funebralis*. They stated that further studies of this nature should investigate salinity-induced changes in those organic and inorganic ions which are major osmotic effectors of tissue fluids. Such a study was conducted by Gilles

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(1972) on three species of pelecypod molluscs, and he found no evidence of osmotic regulation for any of the three species studied. Yet, it does not seem feasible to look to pelecypods for osmotic regulation since most species have the ability to either shut themselves off from the environment or burrow into the substratum if the environment becomes stressful. This author has observed the ability of oysters to remain in air at 10° C for two weeks and then be returned to their normal aquatic environment, to survive and grow. Robertson (1964) suggested that if osmotic regulation does occur within some marine and estuarine mollusc species, it would more likely be found among the estuarine prosobranch gastropods. Osmotic regulation within the true marine forms is not expected since evolutionary adaptation to the stenohaline conditions of the marine environment would favor osmotic conformity rather than osmotic regulation.

The present study was conducted in response to the suggestions of Robertson (1964) and Peterson and Duerr (1969). It investigated salinity-induced changes in major organic and inorganic osmotic effectors of tissue fluids of an estuarine prosobranch gastropod species. The species chosen for study was the common oyster drill, *Urosalpinx cinerea* (Say, 1822), a highly active, predatory animal incapable of closing itself off completely from the environment due to the presence of a siphonal canal. The population from which the test animals were taken occurs subtidally in Great Bay, New Hampshire. The salinity regime of the population ranges from about 5‰ during spring freshets to 30‰ in late summer. Periods of reduced salinities of less than 10‰ during spring may approach four weeks duration (personal observation) depending on accumulated snowfall, ice cover, rate of melting and spring rains.

Materials and Methods

Animals were collected from the field four weeks prior to the start of the study and acclimated in the laboratory to a salinity of 30 ± 0.1‰ and a temperature of 20 ± 1° C. Ambient salinity at the time of collection was 28.6‰ and temperature was 25° C. Test animals were chosen for similarity of size and ranged from 22 to 29 mm in shell length. During the acclimation period and the study, the animals were held in 45 liter acryllic plastic aquaria, maintained at a density of three individuals or less per liter of water and fed small (15–25 mm in shell height) *Mytilus edulis*. Nylon screening, secured just below the surface of the water, prevented the animals from crawling upwards out of the water column. Aerated, standing water was used, and the desired salinities were prepared five hours before use by either dilution of Great Bay water with well water or addition of artificial sea salt (Aquarium Systems, Inc.) to Great Bay water. Atomic absorption analysis of the experimental salinities and ambient Great Bay water showed that the relative proportions of the major inorganic ions did not differ significantly (less than 5% variation) from that of "normal" sea water (see Barnes, 1954). Salinities were checked for accuracy (± 0.1‰) by titration with silver nitrate and adjusted if necessary before use. Osmolalities of the test salinities were determined in a semi-automatic osmometer (Advanced Instruments, Inc., Model 3-W). Half of the water in the aquarium was changed every four days during the extended acclimation period. Aquaria were covered with acryllic plastic tops to prevent evaporative loss of water. The pH of all laboratory prepared water never fell

below 7.1 nor exceeded 7.9; these limits are well within the survival range for *U. cinerea* (Sizer, 1936).

The test animals were divided into two groups at the end of the four week acclimation period. One group was subjected to 2.5‰ sequential increases in salinity up to a final salinity of 40‰. The other group was subjected to 2.5‰ sequential decreases in salinity down to a final salinity of 10‰. Ten animals per salinity were randomly sampled for osmotic adjustment at 120 hours exposure to the test salinities of 40, 35, 25, 20, 15 and 10‰, and the tissues were analyzed to determine osmotic adjustment. The remaining animals were then transferred to the next salinity in the series. Exposure time to each of the inbetween salinities (37.5, 32.5, 27.5, 22.5, 17.5 and 12.5‰) was 72 hours. Ten animals were removed from the acclimation salinity of 30‰ just prior to the start of the study, and the tissues were analyzed for initial "osmotic condition."

Osmotic adjustment was determined by recording changes in tissue fluid osmolality, percentage water content and tissue levels of chloride, sodium, potassium, ninhydrin-positive substances (NPS) and free amino acids (FAA). All of these analyses, with the exception of FAA, were conducted on individual animals. FAA determinations were carried out on pooled homogenates of the five tissues. The other five tissues were individually analyzed for percentage water content. The mean water content of the tissues was used to convert tissue constituent values for the other five animals from millimoles per kilogram wet tissue to millimoles per kilogram tissue water (molality).

Preparation of tissue homogenates

Animals were cracked out of their shells, the opercula carefully removed and the tissues quickly rinsed in distilled water, blotted on absorbent paper and dried for two minutes under a stream of air at room temperature. During shell removal care was taken to sever only the muscles connecting the body to the columella to prevent excessive tissue damage and bleeding. The wet tissue was weighed to the nearest mg and then homogenized in a volume of doubly distilled water ten times the wet weight of the tissue. Thus, all tissues were identically diluted 11-fold with distilled water on a weight-to-weight basis. Homogenization was by a motor-driven pestle, and the homogenizing tube was suspended in an icewater bath. The homogenates were placed in air-tight vials and stored in a freezer at –20° C until the actual tissue analyses were performed. Just prior to analysis for tissue constituents the homogenates were removed from the freezer, quickly thawed in warm water and centrifuged in a Sorvall high speed, refrigerated centrifuge (Model RC2–B) at 10,000 rpm (RCF = 12,100) for 30 minutes at 4° C. The pellet was discarded, and fractions of the supernatant were taken for each of the specific analyses mentioned earlier.

Prior to each use all glassware used in the analyses was soaked for several hours in a sulfuric acid-potassium dichromate cleaning solution, soaked and rinsed in a doubly distilled water and then inverted and allowed to air dry.

Tissue water content

Animals were removed from their shells and treated as described in the homogenization section with the exception that they were not homogenized or

frozen. The whole tissues were dried in an oven at 90° C for 72 hours, allowed to cool in a desiccator and then reweighed as dry tissues. Water content was calculated as the difference between wet tissue weight and dry tissue weight and expressed as a percentage of the wet tissue weight.

Tissue fluid osmolality

Osmolality of the supernatant was determined in a semi-automatic osmometer (Advanced Instruments, Inc., Model 3-W). Only 0.25 ml of the supernatant was required. Conversion of supernatant osmolality to tissue fluid osmolality was calculated according to the following formula: theoretical dilution factor of tissue water based on mean tissue water content of the other five animals × the osmolality of the homogenate in mOsmoles = tissue osmolality in mOsmoles. The resulting osmolalities were higher than the true tissue fluid osmolalities because of the inclusion of solutes normally not osmotically active (*e.g.,* calcium carbonate granules stored in certain cells of the digestive tract). Also, the ionic coefficient of an electrolyte solution increases as the solution becomes more dilute (Prosser and Brown, 1961).

Chloride, sodium and potassium

A 0.2 ml fraction of the supernatant was diluted to two ml with doubly distilled water and tested for chloride by the titration method of Schales and Schales (1941). Sodium and potassium were determined in a Coleman flame photometer (Model 21) with a Coleman Galv-o-meter attachment (Model 22) according to the procedures developed by Coleman Instruments, Inc., and listed in the instruction manual accompanying the instrument. For sodium a 0.2 ml fraction of the supernatant was diluted to five ml with a 0.02% Flaminox solution (a nonionic detergent), for potassium a 0.2 ml fraction of the supernatant was diluted to ten ml with a 0.02% Flaminox solution.

These methods, while allowing for simplicity and rapidity of analysis, were not suitable for the determination of total chloride, sodium and potassium levels. Some unknown percentage of each element was most probably complexed with tissue proteins or other organics which were spun-down during centrifugation of the distilled water diluted homogenates. However, these three elements occur mostly as free, noncomplexed ions in extracellular and intracellular body fluids (Steinbach, 1962; Oser, 1965). Losses due to centrifugation were considered to be inconsequential to the scope of this study since complexed forms of chloride, sodium and potassium would be osmotically inactive anyway. Deproteinization of the supernatant fractions taken for the chloride, sodium and potassium determinations was obviated by the final dilution of these samples prior to photometric analysis (see Schales and Schales, 1941; Hermann and Alkemade, 1963).

Ninhydrin-positive substances (NPS)

A 0.2 ml fraction of the supernatant was brought up to four ml with 75% ethyl alcohol, mixed well and heated for ten minutes at 80° C to precipitate protein. The homogenate-alcohol mixture was centrifuged for eight minutes at

5000 rpm (RCF = 3020), and the supernatant was decanted and saved. The remaining pellet was washed with two ml of 75% ethyl alcohol, recentrifuged and the washing added to the first supernatant obtained. The combined supernatant (original plus washing) was evaporated to just dryness in a drying oven at 80° C. The residue present in the bottom of the tube was redissolved in eight ml of doubly distilled water and tested for NPS by the method of Troll and Cannan (1953). Absorbance was measured in a Bausch and Lomb Spectronic 20 colorimeter at a wavelength of 570 millimicrons. Several concentrations of L-leucine were used to prepare a standard curve.

Free amino acids (FAA)

A 0.2 ml fraction was taken from each of the five supernatants, and these fractions were combined to yield a one ml pooled sample. A 0.2 ml fraction was then taken from this pooled sample and treated as outlined in the procedure for NPS. The dried residue was dissolved in four ml of a 2.2 pH citrate buffer and analyzed for individual free amino acids in a Beckman automatic amino acid analyzer (Model 120C) using the standard, protein hydrolyzate run (Spackman, Stein and Moore, 1958). Taurine, a sulfonic acid, is included in the FAA category.

RESULTS

Only four animals died during the course of this study. One death per salinity was recorded at the salinities of 40, 35, 22.5 and 15‰. Behavior was "normal" within the salinity range of 20 to 35‰. Animals transferred to the 15 and 40‰ salinities exhibited reduced feeding and crawling activity, but 75 to 80% of the animals were attached to the substratum at the end of 120 hours exposure. The

TABLE I

Mean tissue concentrations and ranges of the individual "osmotic" variables in test animals from the Great Bay, New Hampshire population of Urosalpinx cinerea *for each of the experimental salinities.*

Tissue variable	Salinity (°/oo) and Osmolality (mOsmole)						
	10 °/oo 289 mOsmole	15 435	20 579	25 726	30 879	35 1025	40 1180
Osmolality (mOsmole)	628	787	979	1159	1210	1389	1419
	613–670	761–806	931–1016	1028–1226	1175–1309	1329–1468	1309–1539
Water content (%)	77.5	76.6	74.9	73.1	71.9	70.8	70.3
	75.5–80.0	70.5–81.1	71.6–76.7	70.0–76.2	67.8–76.7	66.7–75.5	69.1–72.2
Chloride*	105.7	112.7	152.1	212.6	234.9	291.8	309.0
	95.6–113.4	96.9–126.8	138.8–159.9	195.8–234.3	209.2–263.5	260.4–311.3	272.9–351.4
Sodium*	97.9	119.8	152.1	209.8	218.1	222.1	244.8
	81.4–109.6	115.2–127.9	126.5–173.0	194.1–240.6	192.7–244.8	204.0–243.4	226.9–263.0
Potassium*	69.5	73.3	78.8	91.0	93.6	99.8	100.9
	66.4–71.8	70.0–74.5	71.8–90.0	85.4–94.7	88.2–105.0	94.5–103.9	97.6–107.7
Total FAA*	28.9	42.6	47.9	103.2	127.0	124.1	138.8
NPS*	54.1	62.1	55.4	123.3	130.6	151.3	163.9
	41.6–66.5	56.3–73.3	48.6–68.6	118.5–129.0	107.2–170.9	130.3–167.5	147.8–184.8
Total FAA as a percentage of NPS	53.5	68.7	86.4	83.7	97.3	82.0	84.7

* mM/kg tissue water.

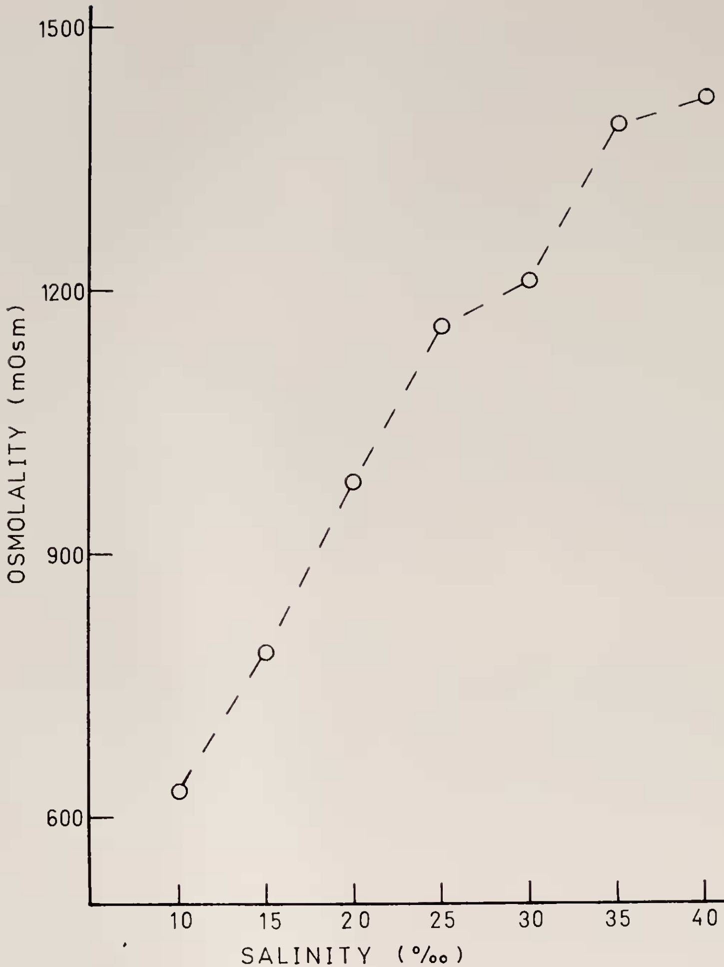

FIGURE 1. Salinity-induced changes in the mean tissue fluid osmolality of test animals from the Great Bay, New Hampshire population of *Urosalpinx cinerea.*

animals transferred to the 10‰ salinity displayed an immediate stress response and exhibited no feeding activity. However, by the end of 120 hours exposure at 10‰ approximately 40% of the animals were attached to the substratum and all exhibited a complete withdrawal into the shell when subjected to a tactile stimulus.

Changes in mean tissue fluid osmolality and mean tissue concentrations of chloride, sodium and potassium followed the direction of the salinity changes (Table I; Figures 1 and 2). These variables increased with increasing salinity and decreased with decreasing salinity. Changes in mean tissue water content approximated an inverse, linear relationship with salinity (Table I; Fig. 3).

Changes in tissue concentrations of FAA followed the direction of the salinity changes with one exception; the FAA concentration at the 35‰ salinity was

2.9 mM/kg tissue water less than the FAA concentration at the 30‰ salinity (Table I; Fig. 4). Changes in mean tissue concentrations of NPS followed the direction of the salinity changes between 25 and 40‰, but the mean NPS concentration at 20‰ was 6.7 mM/kg tissue water less than that at 15‰ (Table I; Fig. 4). The mean NPS concentration at 10‰ was only 1.3 mM/kg tissue water less than the 20‰ concentration.

Differences in magnitude between the 40‰ and 10‰ mean tissue concentrations of the variables investigated are as follows: tissue fluid osmolality 2.3; chloride, 2.9; sodium, 2.5; potassium, 1.4; FAA, 4.8; NPS, 3.0 and 1.1 for percentage water content. It is apparent from this list that FAA exhibited the greatest relative change within the salinity range of this study, while the percentage of water content exhibited the least relative change. Of the inorganic ions investigated, potassium showed the least change with salinity and chloride the most change. The total difference between the 40‰ and 10‰ mean tissue concentrations

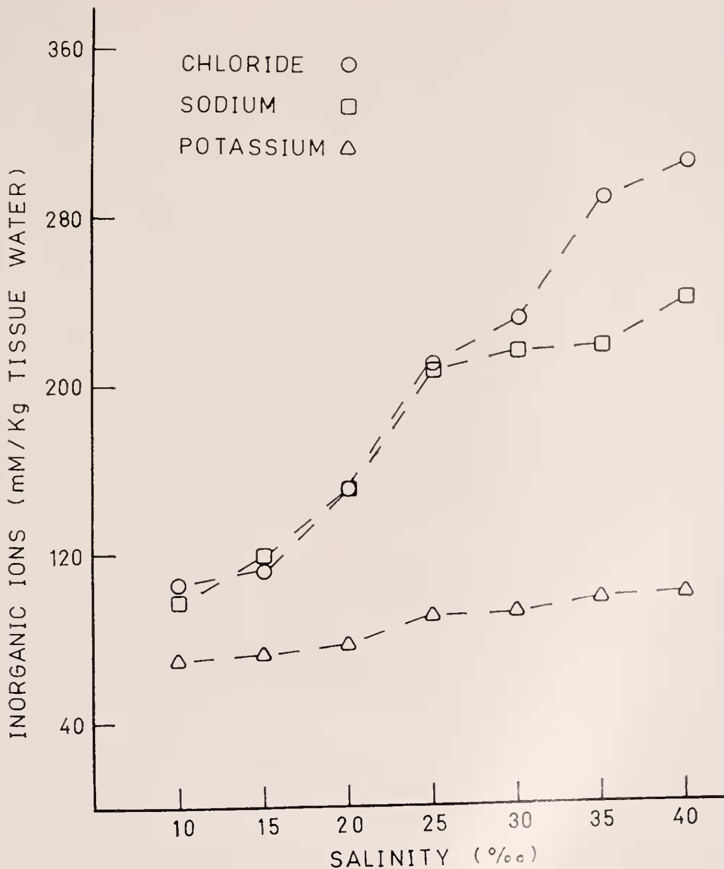

FIGURE 2. Salinity-induced changes in the mean tissue concentrations of chloride, sodium and potassium of test animals from the Great Bay, New Hampshire population of *Urosalpinx cinerea*.

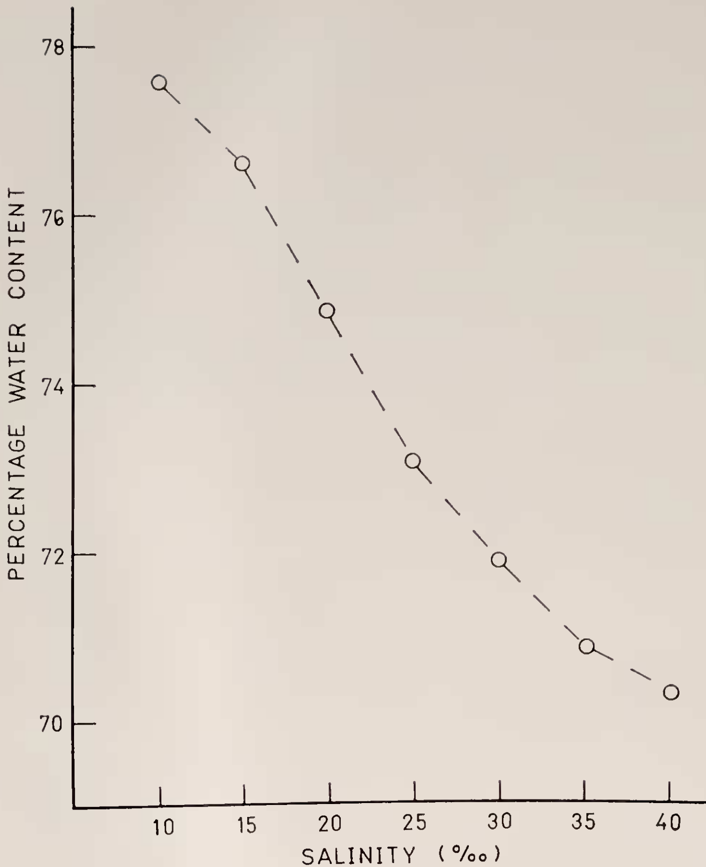

FIGURE 3. Salinity-induced changes in the mean tissue water content of test animals from the Great Bay, New Hampshire population of *Urosalpinx cinerea.*

of potassium was 31.4 mM/kg tissue water, whereas these differences for chloride and sodium were 203.3 and 146.9 mM/kg tissue water, respectively. Mean tissue chloride concentrations at the salinities of 25, 20, 15 and 10‰ were less than 8 mM/kg tissue water different from the corresponding sodium concentrations. But, the chloride concentrations at the salinities of 30, 35 and 40‰ were, respectively, 16.8, 69.7 and 64.2 mM/kg tissue water greater than the corresponding sodium concentrations.

FAA contributions to the mean tissue concentrations of NPS varied from 53.5 to 97.3%. The largest percentage contribution occurred at the acclimation salinity of 30‰. The lowest contributions occurred at the 15‰ (68.7%) and 10‰ (53.5%) salinities. Percentage contributions for the other four salinities were similar and ranged between 82.0 and 86.4%. Taurine was the most abundant amino acid at all experimental salinities (Table II) and comprised from 36.6 to 52.7% of the FAA pools. Other amino acids present in relatively consistent high

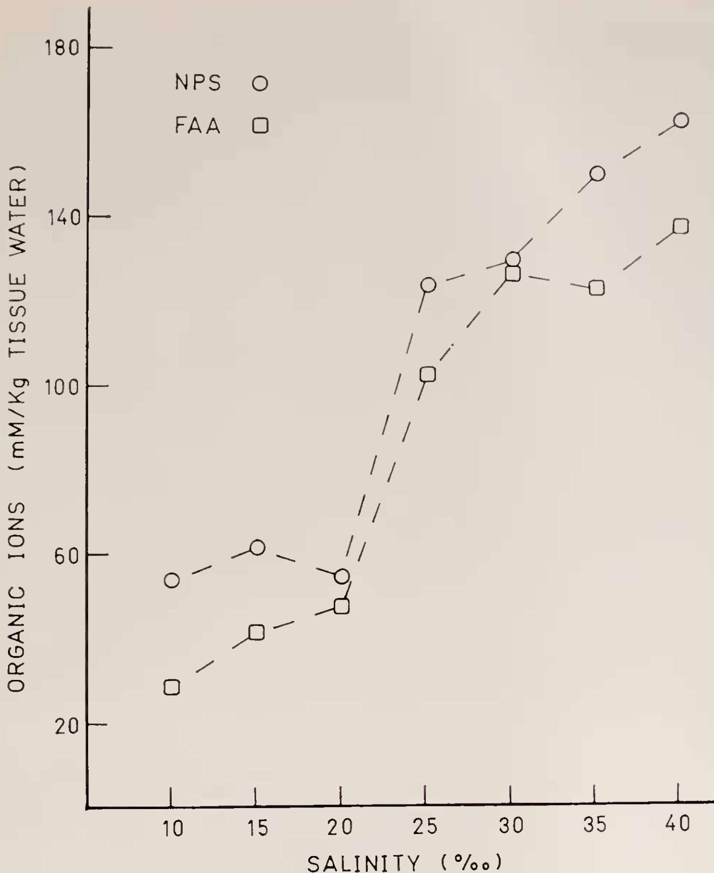

FIGURE 4. Salinity-induced changes in the mean tissue concentrations of total free amino acids (FAA), including the sulfonic acid taurine, and ninhydrin-positive substances (NPS) of test animals from the Great Bay, New Hampshire population of *Urosalpinx cinerea.*

amounts were aspartic acid, alanine and glycine. These three amino acids plus taurine comprised from 59.5 to 75.7% of the total FAA pools.

Discussion

Because measurements of all tissue variables were conducted on whole tissue homogenates, it is impossible to discern the intracellular and extracellular distribution of the individual ionic "species". However, it is known that inorganic ions, primarily chloride and sodium, are the major osmotic effectors of the blood of marine and estuarine invertebrates (including molluscs), while FAA and other small organic ions are major osmotic effectors of the intracellular fluids (Potts and Parry, 1964; Robertson, 1964; Florkin, 1966; Schoffeniels, 1967). Thus, the intracellular and extracellular fluids are isosmotic with each other but differ in the types and concentrations of ions responsible for the respective osmolalities.

Salinity-induced variations in tissue concentrations of all osmotic ions investigated and tissue fluid osmolality were greater than those expected due to changes in tissue water content alone. Osmotic adjustment to the various test salinities must have included not only changes in tissue water content but also external-internal fluxes of the ions themselves. Ionic exchanges between the external medium and internal body fluids would "buffer" the degree of tissue hydration or dehydration and allow the cells to become isotonic with the external medium after an initial period of osmotic and ionic adjustment. In this regard severe tissue hydration or dehydration did not appear to be a problem confronting the test animals, and changes in tissue water content were much less than would be expected if the animals had acted as perfect osmometers with no mechanism of volume regulation. Test animals at the low salinities of 15 and 10‰ did not appear swollen and, upon tactile stimulation, could retract body and foot completely into the shell. At this point it is worth mentioning that no deaths occurred at the minimum test salinity of 10‰ during this study, and that the 11 "extra" animals remaining at the 10‰ salinity were held at this salinity for an additional two weeks before the first death was recorded. Surprisingly, one (or perhaps two) of these animals deposited two egg cases during this additional holding period at the 10‰ salinity. The viability of these egg cases was not investigated.

The nearly linear relationship between tissue chloride concentration and salinity and the large order of magnitude of change in tissue chloride over the range of test salinities shows that chloride ions were freely exchangeable between the external medium and the internal milieu. Robertson (1949) investigated ionic regulation in six species of marine molluscs, including two species of prosobranch

TABLE II

Tissue concentrations of individual free amino acids in test animals from the Great Bay, New Hampshire population of Urosalpinx cinerea *for each of the experimental salinities. Concentrations are in mM/kg tissue water; tr indicates traces; dashed lines, not detected.*

Amino acid	Salinity (‰)						
	10	15	20	25	30	35	40
Alanine	1.77	2.74	2.74	9.17	10.56	9.17	15.28
Arginine	0.63	0.79	tr	0.35	1.49	0.26	0.92
Aspartic acid	4.01	4.42	7.40	9.31	11.45	9.56	10.04
Cysteic acid	1.61	2.16	6.28	tr	2.51	3.05	2.30
Glutamic acid	1.87	2.69	3.62	5.23	5.09	3.75	3.87
Glycine	1.52	2.51	0.90	8.62	14.56	11.58	14.38
Histidine	—	—	tr	0.23	0.39	tr	0.64
Isoleucine	0.49	0.18	0.36	1.56	1.62	1.68	1.98
Leucine	0.62	0.46	0.44	2.34	2.37	2.24	2.62
Lysine	1.19	1.25	0.50	5.35	4.31	3.78	3.44
Phenylalanine	—	—	tr	0.43	0.45	0.39	0.32
Proline	—	tr	tr	4.15	3.53	3.06	7.04
Serine	0.45	1.32	1.81	4.97	4.82	4.47	6.17
Threonine	0.26	0.85	1.04	2.58	3.63	3.85	4.31
Tyrosine	—	—	tr	0.39	0.35	0.37	0.34
Valine	—	0.42	4.20	2.40	2.68	2.74	3.40
Taurine	14.49	22.47	17.52	45.96	57.07	63.71	61.27

gastropods, and found that they did not regulate blood chloride levels. His data showed that the blood chloride concentrations of these species were within ±1% that of the external medium. The order of magnitude change in tissue chloride concentration in the present study was 2.9 between the extreme salinities of 10 and 40‰. This represents a rather large change, especially since the measurements were made on whole tissue homogenates rather than on just blood samples. Thus, it is concluded that the experimental animals did not regulate blood levels of chloride but that the blood chloride concentrations were, for all practical purposes, identical to that of the external medium over the range of test salinities employed.

Tissue sodium showed the same relationship with salinity as did chloride between the salinities of 10 and 25‰ but between 25 and 30‰ the slope of the linear relationship decreased sharply and remained decreased up through the 40‰ salinity. These data suggest a nonregulation of blood sodium levels between 10 and 25‰ and a hyporegulation between 25 and 40‰. Robertson (1949) found a slight (3%) hyporegulation of blood sodium in *Buccinum undatum*, another marine prosobranch gastropod, at a salinity of approximately 32‰ but did not test for any possible variation in blood sodium regulation at other salinities. If, in fact, the experimental specimens of *U. cinerea* did exhibit hypo-ionic regulation of blood sodium at the test salinities of 25 to 40‰, then there must have occurred either an increased hyper-ionic regulation of some other cation(s) or an increased hypo-ionic regulation of some anion(s) or both if the electro-chemical balance of the blood was to be maintained.

Although tissue potassium varied linearly with salinity, the order of magnitude change between consecutive experimental salinities was small. Much of the change in tissue potassium concentration with salinity can be accounted for by change in tissue water content. Potts (1958) found that most of the salinity-induced change in potassium concentration of *Mytilus edulis* adductor muscle could be accounted for by change in tissue water content. Marine molluscs studied to date concentrate potassium at levels several times greater than that of the surrounding sea water (Potts and Parry, 1964). The internal-external distribution of potassium is intracellular > extracellular > sea water. In the present study tissue potassium concentrations were several times greater than those of the respective experimental salinities. Data presented by Hayes and Pelluet (1947) showed that the potassium concentration of *B. undatum* foot muscle was approximately 11-times greater than that of the blood. Robertson (1949) found that *B. undatum* exhibited a large (42%) hyper-ionic regulation of blood potassium compared to the external medium. In light of the above information, it is suggested that the experimental specimens of *U. cinerea* exhibited a substantial degree of hyper-ionic regulation of tissue potassium, and that the exchange of potassium ions between the internal milieu and external medium was quite limited over the entire range of test salinities. Such a mechanism of nearly steady-state hyper-regulation would greatly limit the osmotic function of potassium to changes in external salinity. Gilles (1972) observed the blood and perivisceral potassium levels of two species of marine bivalves to remain nearly constant over a salinity regime of 100 to 25% sea water and has also questioned the osmoregulatory importance of potassium regulation in marine molluscs.

FAA concentration showed a continuous decrease with salinity between the 30 and 10‰ salinities, whereas NPS concentration leveled off between 20 and 10‰.

This resulted in a substantial decrease in FAA contribution to the NPS pools at the 15 and 10‰ salinities and suggests that other, unidentified nitrogenous compounds were major contributors to intracellular osmolality at these low salinities. The compounds involved may be the same as or similar to the dialyzable, unidentified compounds found in *Mytilus edulis* (Briceteux-Grégoire, Duchâteau, Jeuniaux and Florkin, 1964) and *Tegula funebralis* (Peterson and Duerr, 1969). It is worth noting that the highest FAA contribution occurred at the acclimation salinity of 30‰. It is unknown if other FAA contributions would have approached this maximal level if the period of exposure to the other salinities had been extended.

The relative abundances of the individual FAA obtained in this study are in partial agreement with those for other marine prosobranch gastropods. Simpson, Allen and Awapara (1959) found taurine, alanine, arginine, aspartic acid, glutamic acid and glycine to be the six most abundant FAA in *Thais haemastoma, Polinices duplicata* and *Oliva sayana*. Peterson and Duerr (1969) found a similar FAA distribution in *Tegula funebralis*. The major difference between the FAA results of the present study and these other studies is the relative concentrations of arginine. In the present study arginine concentrations were consistently low, whereas these other investigators found arginine to be a major component of the FAA pools of the above four species. The low arginine values are unexpected, since one would suppose arginine phosphate to be present in the muscles. Several, preliminary "long column" runs in the amino acid analyzer revealed the presence of ornithine, a known metabolite of arginine hydrolysis (Tschiersch and Mothes, 1963), at levels up to 5 mM/kg tissue water in animals held at the acclimation salinity of 30‰. Thus, it is conceivable that much of the arginine was converted, through hydrolysis, to ornithine and other metabolites. This would account for the low levels of arginine obtained.

The large salinity-induced changes in FAA concentrations observed in this study show that these organic compounds were major components of the intracellular osmolality of the test animals. Robertson (1964; based on data of Potts, 1958) calculated that the FAA pool comprised 47.6% of the major osmotic constituents in *Mytilus edulis* fast adductor muscle in full-strength sea water and 54.0% in half-strength sea water.

The tissue fluid osmolality, chloride, sodium, potassium and FAA data of this study demonstrate that the laboratory population of *U. cinerea* did not exhibit anisosmotic regulation within the range of test salinities employed. The slight decrease in total FAA concentration between the 30 and 35‰ salinities does not warrant a contention of attempted intracellular anisosmotic regulation, since FAA constitute only a portion of the total intracellular osmotic effector pool. It is postulated that the Great Bay population of *U. cinerea* does not maintain its euryhaline survival status through an osmoregulatory mechanism. Rather, the population has probably adapted physiologically to withstand dilution of its body fluids during spring conditions of low salinities.

Summary

Individuals from a subtidal, estuarine population of the common oyster drill, *Urosalpinx cinerea* (Say, 1822), were brought into the laboratory and tested for

osmotic adjustment to changing salinity. Tissue variables monitored at seven experimental salinities ranging from 10 to 40‰ were tissue fluid osmolality, chloride, sodium, potassium, free amino acids (FAA), ninhydrin-positive substances (NPS) and water content. The results of this study demonstrate that the test animals did not exhibit anisosmotic regulation at any of the experimental salinities. However, the data do suggest a high degree of hyper-ionic regulation of potassium at all experimental salinities and a hyporegulation of sodium between the 25 and 40‰ salinities. Taurine, aspartic acid, alanine and glycine were the four FAA present in relatively consistent high amounts. These four amino acids comprised from 59.6 to 75.7% of the total FAA pools.

It is postulated that the population does not maintain its euryhaline survival status through an osmoregulatory mechanism. Rather, the population has probably adapted physiologically to withstand dilution of its body fluids during spring conditions of low salinities.

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